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THE FATE OF KILLED NONHEMOLYTIC STREPTOCOCCI INJECTED INTO THE BLOOD, AND THE RESULTING CELLULAR CHANGES

WITH PLATE 1

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I. THE GENERAL RESULTS

In connection with work on the fate of India ink and of tubercle bacilli when introduced into the blood, the results of which are yet to be published, I have studied also the fate of nonhemolytic streptococci and the resulting cellular changes. On account of the difference in the reactions that may follow the introduction of bacteria into the blood, depending on whether the bacteria are virulent or nonpathogenic, it is impossible to determine beforehand what the fate of a given bacterium may be in a certain animal on the basis of what we know at present about these questions.

Wissokowitsch,[1] as early as 1886, showed that both pathogenic and nonpathogenic bacteria introduced into the blood either decrease greatly or disappear wholly from the circulation in a short time, and that the bacteria are stored in the liver, spleen and marrow, partly in the endothelial cells, partly in the capillaries. Others (Werigo,[2] Arima,[3] Römer,[4] Filatow[5]) also found that these organs, especially the liver and spleen, were of great importance in ridding the body of bacteria. Kyes[6] ascribes the immunity of the pigeon to the pneumococcus to the phagocytic action of the endothelial cells of the liver and spleen rather than to the action of the fluids of the body or to its high temperature. Bartlett and Ozaki[7] and Hopkins and Parker[8] observed that certain bacteria introduced into the blood accumulate in the vessels of the lung rather than of the other organs. The work mentioned, as well as that of others, will be discussed more in detail later.

I selected a nonhemolytic streptococcus because it is easy to recognize the cocci in the tissues and the blood, and because when thoroughly broken up into its simple form, it does not tend to form

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[1] Ztschr. f. Hyg. u. Infectionskh., 1886, 1, p. 2.

[2] Ann. d. l'Inst. Pasteur, 1894, 8, p. 1.

[3] Mitteilungen d. Med. Gesellsch. zn Osaka, 1910, 9, p. 962; Arch. f. Hygiene, 1911, 73, p. 265.

[4] Arch. f. Augenheilkunde, 1906, 55, p. 313.

[5] Arch. f. Augenheilkunde, 1911-12, 70, p. 185.

[6] Jour. Infect. Dis., 1917, 18, p. 277.

[7] Jour. Med. Res., 1917, 35, p. 466.

[8] Jour. Exp. Med., 1918, 27, p. 1.

aggregates. Killed cocci were used in order to make the process as simple as possible by eliminating growth and the formation of toxic substances.

A 24-hour growth of the streptococcus in 180 c c of ascites dextrose broth was collected by centrifugation, washed three times with salt solution, and suspended in 50 c c of this solution. This suspension was shaken violently for several hours when 40% formaldehyd was added in the ratio of 1%, the shaking repeated and the suspension left to stand for at least 18 hours. The cocci were collected once more and washed several times, then suspended in 2 c c of salt solution for every 10 c c of the original culture, and shaken again. The suspension was then left standing and the upper part removed from time to time. In this way a suspension of single cocci was obtained with but few chains of 3 members. About 2.5 c c were injected into the jugular vein of guinea-pigs weighing about 300 gm. In a few cases the injection was made into the left ventricle of the heart.

After the injection the animals were killed, one immediately and then at intervals of 5, 10 and 30 minutes, and in 1, 2, 6, 12, 48, 72 and 96 hours.

The organs and tissues were removed immediately after death and placed in Zenker's fluid. The marrow, which was the last to be removed, sometimes did not reach the fluid until after 20 to 30 minutes. Five micron sections were made and stained with Delafield's hematoxylin and eosin and a modification of the Gram-Weigert stain, using the following method:

1. Paraffin removed in xylol.
2. Absolute alcohol, then 95% alcohol, then in 1% solution of iodin in 95% alcohol for about 10 minutes, and then in 1% sodium carbonate.
3. Running water for 10 minutes followed by distilled water.
4. Hematoxylin 8 to 10 minutes.
5. Destained in 1% hydrochloric acid in 70% alcohol until blue color almost vanished.
6. Running water for 30 minutes, then in 0.2% aqueous solution of eosin for one minute, followed by running water for 10 minutes or more.
7. Three per cent. carbol gentian violet 30 minutes in incubator, then washed with 2% carbolic acid solution in water and placed in Lugol solution for 1 to 2 minutes.
8. Washed with water, blotted with filter paper, cleared with anilin oil and xylol equal parts followed by several changes of xylol; mounting in xylol balsam.

The method is a modification of Czaplewski,[9] in which phenol solution is used instead of anilin oil water.

Special attention was given to the relation between the cocci and the cells, particularly the endothelial cells, in all the organs and tissues, but in spite of the great care and extent of examination the results seem relatively meager. Briefly stated, the cocci were found in cells for only a short time after the injection, and almost exclusively in the endothelial cells of the liver, the spleen, and in the so-called spleen cells, and the leukocytes. The polymorphonuclear pseudo-eosinophil leukocytes seemed to be very active so that within 5-10 minutes after the injection practically all the cocci seemed to have been taken up by the leukocytes, particularly those in the capillaries of the lungs, but even immediately after injection cocci were found in the endothelial cells of the liver and spleen; it is possible that these cells were phagocytically active even after the death of the animal. Practically no phagocytosis was observed on the part of the endothelial cells of the other organs and the tissues, including the brain and spinal cord as well as the large vessels. In the lungs, suprarenals, cortex of the kidney and the glomeruli suggestions of phagocytosis were seen sometimes, and in that case only one or two cocci were found in each cell. It was rather surprising that marrow, the endothelial cells of which take up India ink granules actively as well as particles of blood pigment, showed no distinct phagocytic action for the cocci, only a few of which surprisingly enough were ever found in the marrow. As stated, the leukocytes in the blood stream took up cocci quickly and in large numbers, the large mononuclears in lesser degree, and in films made before death leukocytes with cocci in them could be found as long as 3 hours after the injection, but only rarely after that time. Free cocci could be found in the blood for only about 30 minutes after the injection, mostly entangled in masses of platelets. Within the cells the cocci seemed to lose rapidly their tingibility and to become smaller, finally forming small red granules before complete disappearance, which usually took place within 72 to 96 hours. The liver seemed the most active in the removal of the cocci, the spleen being next in order. Within a short time after injection definite cellular reactions developed in these organs as well as in the marrow, the lungs, the kidneys and the suprarenals. The bone marrow showed great increase in the number of polymorphonuclear leukocytes as soon as 10 minutes after the injection, even though hardly any cocci were present; later these leukocytes diminished in number while transitional forms seemed to increase, being like the myelocytes rather larger than normally, the nuclei coarser. The increase in leukocytes in the marrow reached its maximum in about 30 minutes after the injection to fall again after about 3 hours followed by a second wave of increase in about 3 hours more. The megakaryocytes seemed to undergo degeneration with pyknosis, some showing mitosis. The endothelial cells of the liver would vary in size and staining; a specially large type with a round or oval nucleus and a large amount of cytoplasm would arise and project into the lumen of the capillary as if about to fall off. Such cells were actively phagocytic. In the spleen a great number of plasma cells appeared soon after the injection, arising from lymphocytes, the nuclei of the two kinds of cells appearing alike. There also appeared in the spleen a degenerate type of cell from pycnosis or karyorrhexis of lymphocytes, which was found in the pulp as well as in the malpighian bodies. The endothelial and reticular cells seemed large and swollen. In the kidney and suprarenals the endothelial cells seemed large also, with increased chromatin, and in some cases there was interstitial proliferation. In the lungs the endothelial cells seemed to react in the same way and to give rise to some of the mononuclear cells in the lung. Similar changes as in the spleen were found in lymph nodes in various parts. In the submucosa of the stomach, and especially of the intestines as well as in the uterus, the number of plasma cells appeared to increase greatly after the injection.

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[9] Schmorl. Untersuchungensmethoden, 1914, p. 311.

Lungs.—Here the bacteria accumulate first in the course of their distribution after being injected into the jugular vein. Almost immediately innumerable cocci were found in the lungs, often accumulating in the capillaries, sometimes to a marked degree, the capillaries dilating from local toxic action. Even at this time the cocci were mostly found within polymorphonuclear leukocytes, a few being seen also in mononuclear cells and in endothelial cells. After 10 minutes the number of polymorphonuclears seemed to have increased and almost all the cocci had been taken up. In the course of the next 20 minutes or so the leukocytes became scattered in the circulation and the number of cocci seen smaller; after 2-3 hours the bacteria became few, and at the end of 24 hours the condition seemed quite normal.

Werigo was the first to observe that bacteria introduced into the blood stream accumulate first in the lungs whence they are transported elsewhere by leukocytes. He injected rabbits with anthrax bacilli. He says that it is evident that in these early stages after injection the lungs represent a source of infection of the circulating blood by the bacteria. Arima found by cultural methods that after injection of colon and typhoid bacilli and of staphylococci the number of organisms in the lungs decrease rapidly in the course of the first 3 hours. He thinks that the diminution of bacteria in the lung is due to the bactericidal action of the blood on one hand and to transportation elsewhere by the blood on the other hand. Bartlett and Ozaki, who injected staphylococci into the left ventricle in dogs, also found by microscopic study that the number of cocci diminished greatly during the first 20 minutes after the injection. At first the cocci were free in the capillaries, but in the course of 10 minutes or so they were taken up by leukocytes, being either destroyed quickly or carried to other parts, principally the liver and spleen. Hopkins and Barker, working with hemolytic streptococci, were impressed with the streptococcidal action of the lung tissue, and the details of their work will be referred to later. All observers seem to agree that bacteria injected into the blood accumulate first in the capillaries of the lung, where they become subject to phagocytosis in a short time so that in 30 minutes or so most of them are removed, the lungs being apparently quite free in about 3 hours, until, according to Werigo, bacteria may begin to accumulate again in the lungs. In the experiments just cited living organisms were used, and it might be assumed that different results would be obtained with dead organisms, but my results agree very well with those of the aforesaid experiments. In my experiments endothelial and mononuclear cells took more part in the action than in Werigo's experiments with anthrax bacilli. Bartlett and Ozaki, as well as Hopkins and Parker, noted similar action on the part of these cells. Hopkins and Parker concluded that streptococci taken up by the cells in the lung were killed within 5-8 hours although they remained visible in the sections after a number of days, the destruction being due to the action of living cells as extracts of the lung had no destructive effect. In my experiments, however, there seems little doubt but that the bacteria disappeared from the lungs principally as the result of transport by leukocytes to other places and also as the result of digestion within the phagocytes. I would emphasize especially the fact that the cocci on injection accumulate in the lung capillaries even when the injection of carefully made suspensions was done slowly and gradually. Bull[10] observed that certain bacteria, when injected intravenously into rabbits, would be agglutinated while others would not unless the animals were previously injected with a corresponding antiserum. He believes that the agglutination in the blood is favorable to the elimination of the organisms as the leukocytes can take up large numbers of bacteria more readily. In my experiments, the cocci seemed to accumulate loosely without being first agglutinated, and it seemed to me that this resulted in a measure from the failure of the suspension to mix well with the blood, the bacteria gathering first in the lungs and leading to the accumulation of leukocytes, some of course being carried directly through the lung into the blood. As the bacteria form collections in the blood in the lung the distribution later to other parts, especially the liver, is irregular and many phagocytic endothelial cells may be loaded with bacteria while others have few or none. There may be differences in phagocytic activities also, but that would hardly account for the irregular distribution.

When the same quantity of bacterial suspension that was injected into the jugular vein was introduced slowly into the portal vein and the animal killed five minutes later, the appearances in the liver and the lung were quite the reverse of the appearances after jugular injection. Not only did more endothelial cells in the liver contain cocci, but many polymorphonuclear leukocytes appeared also in the vessels of the liver, while in the lung there were hardly any changes except that some endothelial and mononuclear cells containing a few cocci were present.

Bartlett and Ozaki found that bacteria injected into the left ventricle gathered in large numbers in the lung. I made careful comparison in 3 guinea-pigs, in 2 of which the coccal suspension was injected into the left ventricle, the animals being killed 5 and 10 minutes later, while a third animal received injections into the jugular vein and killed after 10 minutes. There were few cocci in the lungs of the animals that had received injections into the left ventricle as compared with the number in the animal receiving injections into the jugular vein; in the former animals the liver contained the largest number of cocci.

It may be concluded that the bacteria accumulate in the lungs after intravenous injection largely as the result of mechanical influences, notably the difficulty of dispersing the bacteria in the blood, and the small size of the capillaries, to which must be added the secondary accumulation of leukocytes. As shown in my work with India ink, the granules seem to adhere first to the surface of the phagocytes, but the lungs do not contain as many active phagocytic cells as the liver and spleen. This is another reason why the accumulation of bacteria in the lungs is ascribed largely to influences of a mechanical nature.

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[10] Jour. Exper. Med., 1915, 22, p. 457, 475, 484.

Table 1 shows the distribution of polymorphonuclear leukocytes in the organs of four normal guinea-pigs, the number of phagocytic endothelial cells and leukocytes in relation to the total number of such cells at various periods after the injection as well as the total number of phagocytic cells and of leukocytes in all the organs examined. While it might have been more desirable to count the cocci, this seemed to be impracticable because of the formation of lumps and the development of changes so that one coccus could not be distinguished from another, and on this account the average number of cells containing cocci was counted. The figures in the table give the average number of cells in each microscopic field as determined by counting 90 fields from at least 3 different sections, all 5 mikrons in thickness, in each case. Ocular 4 and oil immersion objective 1.8 were used. In the liver the interior of the acini, excluding Glisson's capsules and the central vein, was studied. In the spleen the pulp only was examined. In the case of the marrow no attempt was made to classify the cells definitely, most of them being polymorphonuclears. In order to count all the leukocytes in the marrow the fields were divided into 4 areas and the cells enumerated in 1 area multiplied by 4. While the figures are not to be regarded as exactly representing the numbers of phagocytic and other cells, they represent the periodical variations in the different organs far more accurately than statements based on general observations could.

Table 1 shows that in the liver there are more phagocytic endothelial cells after 5 minutes than immediately after the injection; then the number falls to increase again, but after 3 hours the number again falls and 48 hours after the injection almost all phagocytic endothelial cells have disappeared. Phagocytic polymorphonuclear leukocytes increase steadily for about 3 hours after the injection, the total number of leukocytes also increasing; after that the number of phagocytic leukocytes decreases gradually; the total number of leukocytes also begins to fall after the third hour, the normal number being reached in 72-96 hours. It should be noted that the number of phagocytic endothelial cells reached its maximum earlier than the leukocytes and fell off earlier also.

In the case of the lungs, the polymorphonuclears which had accumulated here began to disperse about 30 minutes after the injection and in from 1-3 hours most of them had left the lung, going probably mostly to the liver and the spleen, thus explaining the gradual increase in the number of these cells in these organs up to the third hour. At the same time leukocytes no doubt were attracted to the liver by the accumulation of detritus from cocci and cells. Hence the endothelial cells in the liver receive cocci that are free in the blood and that are liberated from leukocytes and other cells as they degenerate and fall to pieces.

As pointed out, bacteria may be destroyed rapidly in phagocytes. Werigo noted that anthrax bacilli began to disintegrate within phagocytes 7½ minutes after injection. At the same time some endothelial cells, as well as leukocytes, disintegrate during this process, and in the case of endothelial cells younger

TABLE 1

Distribution of Leukocytes and Phagocytic Cells in the Organs After Injection of Killed Streptococci

	Lung	Liver				Spleen				Bone-marrow		Kidney		Adrenal		Muscle	Total No. of Leukocytes in All Organs
				Leukocytes				Leukocytes		Leukocytes		Leukocytes		Leukocytes			
	No. of Leukocytes	Total No. of Phagocytic Cells	Phagocytic Endothelial Cells	Phagocytic	Total No.	Total No. of Phagocytic Cells	Phagocytic Endothelial Cells	Phagocytic	Total No.	Phagocytic	Total No	Phagocytic	Total No.	Phagocytic	Total No.	Leukocytes	
Normal 1	2.67	.....	.....	.....	0.50	.....	.....	.....	25.2	.....	44.4	.....	0.17	.....	0.22	.....	73.2
2	1.57	.....	.....	.....	0.55	.....	.....	.....	29.2	.....	81.2	.....	0.19	.....	0.18	.....	112.9
3	2.80	.....	.....	.....	0.65	.....	.....	.....	36.4	.....	54.0	.....	0.10	.....	.....	.....	93.9
4	2.17	.....	.....	.....	0.70	.....	.....	.....	31.6	.....	86.6	.....	0.13	.....	0.21	.....	121.9
Average	2.31	.....	.....	.....	0.60	.....	.....	.....	30.6	.....	66.6	.....	0.14	.....	0.20	.....	100.5
40 seconds	9.75	1.62	1.17	0.45	1.33	4.30	2.10	2.20	19.95	1.43	106.2	0.01	0.17	.....	.....	0.23	137.6
80 seconds*	.....	.....	.....	.....	.....	.....	.....	.....	9.50	.....	80.2						
5 minutes	19.15	5.46	5.02	0.44	1.73	2.35	1.25	1.10	4.48	1.03	60.0	0.06	0.12	0.01	0.10	0.08	85.7
5 minutes*	.....	.....	.....	.....	.....	.....	.....	.....	8.55	.....	104.8						
10 minutes	40.10	4.92	4.45	0.47	2.27	5.11	4.72	0.39	7.86	0.64	152.6	0.06	0.21	0.08	0.22	0.15	203.4
30 minutes	18.81	3.96	3.05	0.91	3.17	6.35	5.44	0.91	2.86	0.22	217.0	0.10	0.35	0.23	0.33	0.31	251.8
1 hour	9.85	6.65	5.11	1.54	6.11	7.85	7.19	0.66	6.60	0.30	127.0	0.05	0.47	0.03	0.15	0.35	150.6
3 hours	8.65	4.85	2.88	1.97	13.83	10.83	7.50	3.33	28.42	0.70	73.2	0.05	0.81	0.73	5.80	0.33	126.0
6 hours	3.77	2.61	1.90	0.71	3.17	7.41	5.55	1.86	41.33	1.45	126.2	0.09	0.76	0.30	2.10	0.25	175.8
12 hours	4.13	2.10	1.73	0.37	2.87	6.66	5.00	1.66	55.55	0.30	101.7	0.0	0.83	0.03	0.90	0.20	165.6
24 hours	2.86	1.49	1.42	0.07	1.91	4.66	3.50	1.16	19.95	0.06	93.3	0.02	0.11	0.0	0.33	0.08	118.5
48 hours	2.80	0.79	0.78	0.01	1.12	1.93	1.91	0.02	10.95	0.0	76.9	0.0	0.16	0.01	0.53	0.12	93.6
72 hours	2.61	0.08	0.08	0.0	0.58	.....	.....	0.0	12.75	0.0	60.9	0.0	0.14	0.0	0.06	0.05	74.5
96 hours	2.35	0.0	0.0	0.0	0.51	0.0	0.0	0.0	13.42	0.0	57.3	0.0	0.11	0.0	0.12	.....	73.8

* Suspension of different strain of coccus injected. All other observations were made with same suspensions of same strain.

cells may take up the cocci thus set free. Werigo found a large number of bacilli in the liver 7½ minutes after the injection, the number dropping suddenly after 10 minutes and then more slowly until about the sixteenth hour when an increase began. My figures show two high points, one after the first 5 minutes, and the second one hour after the injection, the only difference from Werigo's results being the second climax which I have ascribed as due to divergence of bacteria from the lungs. Arima also found a diminution in the number of bacteria in the liver after 5 minutes and 1 hour, but his results were based on cultures. Bartlett and Ozaki gave no observation at 5 minutes or so, but their highest figures for the liver were obtained 10 minutes and 1 hour after the injection, there being a definite drop between. These figures were based on direct counting of the bacteria and correspond closely to my results.

The discussion of the endothelial phagocytosis in the liver would be incomplete if we did not consider also the fact that such cells may escape into the blood as noted by Kiyiono[11] and myself in our work with India ink. However, only few phagocytic mononuclear cells were found in the blood in these experiments even when the blood was drawn directly from the right ventricle. This was true particularly at three hours after the injection. In the earlier stages when leukocytes collect in the lung, endothelial cells naturally might accumulate there also and thus account in some measure for the decrease in the number of such cells in the liver. Later on, after the second climax, when practically no phagocytic mononuclear cells could be found in the blood from the right ventricle, the small number of phagocytic endothelial cells in the liver is best explained by rapid intracellular digestion of the cocci. The table shows, however, the presence of phagocytic cells after this period, though in diminishing number, indicating that all cocci are not digested promptly. After 96 hours the cocci had disappeared from the endothelial cells. In Bartlett and Ozaki's experiments and in Kyes' experiments with staphylococcus and pneumococcus, respectively, the cocci were found to have disappeared at the end of 72-96 hours.

Shortly after the injection definite changes could be made out in the endothelial cells. Werigo noted changes 7½ minutes after the injection and grouped the cells into "simples et compliques," the latter having many nuclei, due to the fact that in some cases at least they had taken up leukocytes. In my sections such cells were not commonly found in the liver, being, however, fairly abundant in the spleen. A large number of cells with round or elliptical nuclei and abundant cytoplasm—Werigo's "simples"—sometimes projected into the capillary lumen. Frequently they contained many cocci. In half an hour and then on, the maximum being reached at the third hour, these cells showed marked changes, assuming various shapes and sizes and a deeply stained nucleus. Later the cells began to return to the normal condition and after 72-96 hours only a few changed cells were present. Degenerated cells, endothelial and leukocytic, were seen, sometimes with only faintly stained nuclei or no nuclei, most of them containing many bacteria.

When we consider that the bacteria in the endothelial cells of the liver reach a high point one hour after injection, it seems clear that several generations of cells one after the other are required to destroy all the bacteria even though they were dead when injected. As noted, some cocci appear to be destroyed much more rapidly than others, due possibly to changes in the bacteria themselves, the older bacteria being perhaps destroyed more quickly than the younger.

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[11] Vitale Karminspeicherung, Jena, 1914, Nisshin-Igaka, 1914, 4, p. 917.

Werigo states that as leukocytes are taken up by larger phagocytic cells (macrophages) the bacteria are transferred to the latter. In regard to the fate of the leukocytes after the delivery of the bacteria he says that most of such leukocytes appear quite normal, evident signs of destruction being observed only rarely so far as observed in the liver; in the spleen, however, the leukocytes taken up by other cells appear to be destroyed. The impression is made that leukocytes engulfed by other cells in the liver may possibly return again to the blood.

We know that the endothelial cells of the liver and spleen are a place of destruction of red corpuscles. Kyes showed that in the pigeon pneumococci are taken up by these hemophagocytes in the liver and spleen. Whatever the fate of healthy leukocytes that may be taken up may be, there is no question but that the dead and dying leukocytes are treated in the same way as red cells and foreign bodies by phagocytic cells, and in that case bacteria such as streptococci within such leukocytes would be passed over to the larger phagocytic cells in a passive manner.

Spleen.—The cocci were taken up by endothelial cells and spleen cells, but always in small numbers only, groups or masses being found but rarely. In the earlier stages the malpighian bodies did not contain any cocci; later cocci appeared in the reticulo-endothelial cells, but altogether the phagocytic activity of these cells was less than that of the endothelial cells of the liver. Immediately after the injection and 5 minutes later the phagocytic cells were only few in number, increasing gradually for about 3 hours when the maximum was reached to be followed by a gradual disappearance, which became complete in 72-96 hours. Phagocytic polymorphonuclear leukocytes also increased in number for about 3 hours when a gradual diminution set in. Comparing the number of phagocytic endothelial and pulp cells with the number of phagocytic cells in the liver, it was found that while the number was greater in the spleen, the number of bacteria taken up was much smaller than in the case of the liver. Werigo also found that bacteria were destroyed more slowly in the spleen than in the liver and he is opposed to Metchnikoff's view that the bactericidal power of the spleen is greater than that of the liver. If we consider the volumes of the two organs it becomes quite clear that the liver is the most important organ for the elimination of bacteria in the blood stream, the spleen being next in order.

In the normal guinea-pig polymorphonuclear leukocytes are present in fairly large numbers in the spleen, approximately 30.8 per field on the average. Immediately after infection of cocci the number fell to about one-fourth to one-tenth of the normal average; after about 6 hours and continuing for about 6 hours more the number increased above the normal, dropping again below normal after 48 hours. The only other organ or tissue in which leukocytes are present normally in such large numbers is the marrow, and the reasons for this accumulation cannot be discussed now. The interesting point is that in the spleen the number of leukocytes decreased immediately after the injection while in the liver the number increased. It would seem as though the leukocytes in the spleen were sent to other organs and especially the lungs. The number of leukocytes taken up by other cells in the spleen was not large enough by far to account for the reduction after injection of streptococci. Such phagocytosis occurs and Werigo traces certain granules found in the macrophagocytes of the spleen to digestion of leukocytes. Mallory[12] and Imamura[13] believe that the granules are rather the remains of lymphocytes. The spleen is an inconvenient place to leave for the leukocytes, on account of the construction of the sinuses and the slowness of the blood current, and there would seem to be a good opportunity for macrophagocytes to take up old and degenerate leukocytes. Add to this, positive chemotactic action and it does not seem difficult to understand that large numbers of leukocytes might be removed from the blood in the spleen during the period between 12-24 hours after injection of cocci, a period when there is no special demand for leukocytes elsewhere, and, as shown in Table 3, no increase of leukocytes in the blood. The question remains whether the leukocytes are destroyed by the activity of the phagocytic cells primarily or due to degenerative processes in the leukocytes. This question cannot be answered definitely, but it seems reasonable that it is principally degenerated cells that are disposed of in this way.

The endothelial and reticular cells began to enlarge 10 minutes after the injection, the nuclei becoming large and the cytoplasm of different cells in places seemingly flowing together so that the distinction between endolethial and reticular cells became more difficult. Mitotic and amitotic cell division increased rapidly and the pulp consequently swelled up. The cells in the malpighian bodies also became enlarged and proliferated, the boundaries of the bodies becoming indistinct. The nuclei of lymphocytes seemed to enlarge, being round or roundish and smooth and the germinal centers consequently increased. Under a low power the field seemed to be composed principally of pulp. After 24 hours and later these reactions subsided gradually and at 72 hours and on to 96 normal conditions as a rule became reestablished. In the course of these changes two special cells made their appearance—plasma cells and a degenerate form to be described more fully later.

Bone Marrow.—There were fewer cocci here than in the liver and spleen, and almost all of them were in polymorphonuclear leukocytes. Singularly enough none were found in the endothelial cells of the marrow which take up other foreign bodies actively. The number of cocci was high immediately after the infection and 5 minutes after; then it decreased gradually up to 30 minutes after the injection when an increase set in which reached its maximum at 6 hours followed by diminution so that at 48 hours practically no cocci could be found. The first stage reached its maximum 5 minutes after the injection and was succeeded by a gradual diminution during the next 30 minutes that may be regarded as the expression of the phagocytic activity of polymorphonuclear leukocytes; it corresponded to what was observed in the spleen except that there seemed to be no diminution in the marrow in the total number of leukocytes, due possibly to the rapid production of leukocytes as indicated by rapid increase of their number. It would seem as if phagocytic leukocytes on reaching the marrow and spleen are sent away again promptly so that there is no accumulation of cocci. The marrow strictly speaking is not actively bactericidal as no cocci were found within the cells proper of the marrow, only within the leukocytes.

[12] Jour. Exper. Med. Med., 1898, 3, p. 611.
[13] Nisshin Igaku, 1916, 6, p. 1871.

The changes in the marrow were remarkable and occurred early. Hyperemia in the earlier stages was so marked that the capillaries seemed to have been broken down. After 5 minutes intensely stained cells were present and after 10 minutes a great many well lobulated polymorphonuclear cells were present in the parenchyma at the same time as mitosis was increased. After 30 minutes, however, the polymorphonuclears had decreased while transitional forms of a large type had increased with evidence in the form of mitotic figures of active cell proliferation. The nuclei of myelocytes and transitional forms were rich in chromatin and stained well. This general condition continued without any further marked accumulation of polymorphonuclears as observed 10 minutes after injection until after 48 hours when a gradual return to normal set in. As observed by Muir[14] under somewhat similar conditions, during the early stages the megakaryocytes became more distinct and soon passed into a condition of pyknosis with deeply stained shrunken nuclei. While the normal type of this cell prevailed in approximately 92% of the cells during the first 30 minutes after the injection, the pyknotic form increased to 60% during the second hour, the number of pyknotic cells thereafter gradually falling, being still more numerous than immediately after the injection even after 96 hours. Karyorrhectic forms were rarely seen, mitosis occasionally. Often a large nucleolus appeared in young megakaryocytes.

Tables 1 and 2 give details concerning the number of leukocytes at different periods after injection. From the fact that the number of leukocytes in the spleen and of phagocytic leukocytes in both the spleen and marrow decreased soon after injection, I conclude that the marrow in the meantime is sending out leukocytes rapidly and that at the same time new leukocytes arise from myelocytes. As shown in table 2, in 4 guinea-pigs killed within 5 minutes after injection (PI) there was some increase in the number of polymorphonuclears, due as I have assumed to rapid transformation of myelocytes, the change in the nucleus taking place as if by a peristaltic action. The large number of well lobulated polymorphonuclears at 10 minutes and later after the injection (P2), with many transitional forms, supports this assumption. There now ensues a diminution (P3) due seemingly to an exhaustion of the marrow with the appearance of metamyelocytes and myelocytes in the blood and a new type of polymorphonuclear (see part 3). From the point of view of mitosis, there is a slight increase during the first 5 minutes after the injection (P'I), this increase becoming more marked at 30 minutes and 1 hour (P'2) when a drop ensues (P'3). During the period between 12 and 24 hours after the injection there is a second increase in the number of mitosis (P'4) ending in gradual return to the normal.

Returning to the number of leukocytes, we note a second increase of polymorphonuclears in 6-12 hours after the injection (P4), due to the new production of myelocytes. After 24 hours the number decreases as the reaction subsides. This reaction would be largely influenced by the number and virulence of invading bacteria and if maintained as the result of the stimulus of living microbes soon no doubt an exhaustion of the marrow would result.

We may regard the preformed polymorphonuclear leukocytes in the marrow as the first reserves for active service in the blood, the myelocytes as the second reserve, and newly formed immature cells as soldiers of the national army. The stages in the reaction in the marrow with respect to cell formation as indicated by the number of mitoses may be divided as follows (table 2):

P'1, the first ten minutes, a preparatory stage.

P'2, 30 minutes to one hour, first stage of active division.

P'3 3 to 6 hours, preparation for second cell division, continued cell production and beginning of new stage in cell production.

P'4, 12 to 25 hours, second stage of cell division.

P'5, 24 to 96 hours, gradual return to normal state.

Interpreted on the basis of the number of polymorphonuclears in the marrow the reaction seems to fall into the following stages:

P1, first 5 minutes, mobilization of leukocytes and formation of new leukocytes from myelocytes.

TABLE 2

POLYMORPHONUCLEAR LEUKOCYTES AND MITOSIS IN MARROW AFTER INJECTION OF NONHEMOLYTIC STREPTOCOCCI IN GUINEA-PIGS

Normal Guinea-Pigs

	1	2	3	4	Average
Number of polymorphonuclears	44.4	81.2	54.0	86.6	66.6
Number of mitosis	0.24	0.36	0.31	0.35	0.32

Guinea-Pigs Injected with Nonhemolytic Streptococci

Time After Injection	Number of Polymorphonuclears		Number of mitosis	
40 seconds	106.4	P1	0.44	P'1
80 seconds	80.2		0.41	
5 minutes	60.0		0.48	
5 minutes	101.8			
10 minutes	152.6	P2	0.71	
30 minutes	217.0		2.20	P'2
1 hour	127.0	P3	3.11	
3 hours	73.2		1.35	P'3
6 hours	126.2	P4	1.71	
12 hours	101.7		2.20	P'4
24 hours	93.3	P5	2.32	
48 hours	76.9		0.73	P'5
72 hours	60.9		0.52	
96 hours	57.3		0.41	

P2, 10 to 30 minutes, climax of transformation of myelocytes into polymorphonuclears.

P3, 1 to 3 hours, beginning exhaustion of preformed and newly formed leukocytes with production of new leukocytes from the first stage of active cell division (P'2).

P4, 6 to 12 hours, accumulation of leukocytes as the result of cell proliferation (P'2, P'3); during the last two stages myelocytes and metamyelocytes may appear in the blood.

P5, 24 to 96 hours, subsidence of reaction.

The mobilization and production of polymorphonuclears in the marrow after the injection of streptococci proceed rapidly. This point will be discussed further in part 4.

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[14] Jour. Pathol. and Bact., 1900-01, 7, p. 161.

II. DEGENERATED CELLS IN THE SPLEEN AND OTHER ORGANS

Besides plasma cells various forms of degenerated cells appear in the spleen. They were seen in small numbers in the earlier stages, but in from 6 to especially 12 hours after the injection they increased rapidly, diminishing again promptly. Such cells occurred free as in macrophagocytes, in the pulp as well as in the follicles, and here more particularly in the germinal centers within reticulo-endothelial cells. The nuclei of these cells varied so much in shape that they can hardly be described, presenting often ring and crescent shapes, and staining from a deep blue to red with many intermediary shades. These cells appeared most abundantly at the time when polymorphonuclear leukocytes were present in large numbers and often subjected to phagocytosis, and some of the degenerate cells may be derived from such leukocytes. Werigo figures bodies like the one in question and regarded them as the remains of polymorphonuclear leukocytes in the interior of phagocytes. Mallory [15] described the same forms in the lymph nodes and other organs in typhoid fever, as well as in the spleen of animals infected with toxic products of the typhoid bacillus. He found them largely within large endothelial cells and traced them to the lymphocytes in process of being digested. Imamura [16] observed similar bodies in the spleen and lymph nodes, especially the germinal centers, and associated with a general hypolymphocytosis, in animals in which hemolysin had been produced; he traced these bodies to lymphocytes and I agree in this view. Such bodies were first described by Flemming,[17] but since that time they have been studied by a number of observers.

In my work it seems clear that these bodies were derived from large and small lymphocytes and lymphoblasts. They may be said to have developed in three ways: (1) by simple hyperchromatosis of the nuclei, (2) disintegration and metamorphosis of chromasomes after mitosis, and (3) disintegration of a compact homogenous nucleus. In the first case there generally results large irregular, ring or crescent bodies, and in the second a collection of small ball-like masses, both these bodies staining red before disappearing completely. The third change seemed to have affected a nucleus just before division. Many authors have observed these forms of disintegration. Helly,[18] Weidenreich[19] and others regard the lymphocytes as the most resistant, and it is difficult to distinguish between cells of this kind that are actually dead and others that are still viable. Certainly the lymphocytes seem to multiply more while in the course of degeneration, the toxic action being evident in the course of cell division. We see it in the mulberry nucleus, in which the chromosomes become spherical directly after mitotic division; in the nevt stage they disintegrate into many roundish masses. This occurred in large as well as small lymphocytes, the larger being more numerous in the germinal centers, the small in the pulp. In other cases the nucleus swells up and bursts at the same time as the cell is taking up cocci. The spleen of a guinea-pig that died from subacute streptococcus infection presented a marked degeneration of the cells in the germinal centers with proliferation of macrophages, and in such areas there was an abundance of tingible bodies.

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[15] Jour. of Exper. Med., 1898, 3, p. 611.

[16] Nisshin-Igaku, 1916, 6, p. 1871.

[17] Arch. F. Mik. Anat., 1885, 24, p. 50; Arch. f. Anat. u. Entwichlysgeschichte, 1885, p. 221.

[18] Wiener klin. Wchenschr., 1904, p. 639.

In the liver of a rabbit that died of chronic streptococcus infection were found many cells having peculiar, spoonlike nuclear masses joining in the center, somewhat like a crysanthemum, evidently incomplete degeneration formed.

Peculiar bodies also occurred in the lymph node, intestinal and elsewhere, as well as in the intestinal villi. Such bodies are found also in normal tissues in small numbers, but as a rule the large pyknotic forms in the spleen and lymph nodes are not present.

III. PLASMA CELLS

As described in part I, plasma cells appeared in the spleen, lymph nodes and the submucosa of the stomach, intestines and uterus, soon after the injection of a killed nonhemolytic streptococcus. In the spleen a fairly large number was present as soon as 5 minutes after the injection, and it was easy to trace their development from lymphocytes. After 24 hours, however, the number of plasma cells fell rather promptly. Normally plasma cells occur in the intestinal submucosa, the omentum, lymph nodes, marrow, submaxillary and other glands as well as in ganglions. Thus Jadassohn and others[20] describe them in the spleen in various mammals including man, but there is no special report on the guinea-pigs in this respect, so for as I can find. In the animals studied so far plasma cells have been found most typically in the rat and mouse: according to Bodora, in man plasma cells may be found either at the periphery of the follicles or in the pulp, these being not wholly typical (pseudoplasma cells). Unna[21] insisted that the plasma cell is a pathologic cell originating from the connective tissue cell, but at the same time he accepted the view of Marschalko that it is a normal cell in the blood-making organs of the mouse. Marschalko urged that the plasma cells in pathologic foci are of hemotogenous origin. Maximow concluded that plasma cells are present sparsely normally and arise in greater numbers in inflammatory conditions. I found on careful examination of the spleen of 5 normal guinea-pigs that a small number of cells were present, resembling very much the typical plasma cell except in respect to the nucleus. In one case plasma cells typical in every respect were found. In the spleen the cells were either grouped or distributed rather irregularly. Usually the cytoplasm did not stain thoroughly and the cells often appeared flat, as described by Maximow. I found a moderate number of such cells in the intestinal wall also. Five minutes after the injection of nonhemolytic streptococci numerous plasma cells would appear, and it is interesting to note that Huebschmann has just found that the spleen is richest in plasma cells in streptococcus infection, especially when it concerns nonhemolytic streptococci.

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[19] Leucocyten u. Verwandte Zellformen, 1911, p. 162.

[20] Jadassohn: Arch. f. Dermat. u. Syph., 1892, 24, p. 216.
Marschalko: Arch. f. Derm. & Syph., 1895, 33, pp. 1 and 241; Centralbl. f. allg. Pathol. u. Anat., 1899, 10, p. 851.
Hodora: Monatschr. f. Prakt. Dermat., 1896, 22, p. 53; Ann. Dermat. Syph., 1895, 6, p. 859.
Unna: Monatschr. f. Prakt. Dermat., 1895, 20, p. 477.
Jovannovics: Ztschr. f. Heilkunde, 1899, 20, p. 159.
Pappenheim: Pappenheim, Virch. Arch., 1901, 166, p. 448.
Maximow: Arch. f. Mik. Anat., 1905-6, 67, p. 680.
Downey: Fol. haemat., 1911, 11, p. 275.
Huebschmann: Verhandl. f. Deutsch. path. Gesell., 1913, 16, p. 110.

[21] Monatschr. f. Prakt. Dermat., 1891, 12, p. 296. Virch. Arch., 1913, 214, p. 320.

Staining method.—The tissues were fixed in Zenker's fluid without acid and the sections stained in hematoxylin and eosin as well as with a modification of the gram-Weigart method. For differentiation in the first case hydrochloric acid alcohol was used and in the second case anilin oil and xylol. By thorough differentiation the hematoxylin sections could be made to show the cocci clearly as well as the chromatin network, while a weaker differentiation would give a more characteristic cytoplasm, the nucleus then being stained more diffusely. With the Gram-Weigert stain only the nucleus and the chromatin in dividing cells would be stained. Perhaps the best demonstration of plasma cell was obtained by using both these methods on the same sections. Unna's polychrome methylene blue and the Unna-Pappenheim stain were also useful.

In the spleen plasma cells were found mostly in the pulp, often in small masses. In the malpighian bodies they usually appeared scattered about and in the germinal center were found both large and small types. They were not present where the lymphocytes were packed together tightly. Usually these plasma cells arranged themselves so as to form some kind of a lumen or space in their midst. The amount of cytoplasm was not always abundant and the shape more irregular than oval or round, due it would seem largely to the spaces which the cells occupied.

Type of Cell.—In the spleen the cell was mostly small (Marschalko's type), although the nucleus often showed only a deeply stained network or small granules rather than a number of peripheral chromatin bodies. Larger types occurred in small numbers, and the cytoplasm was usually rather thin, staining lightly as a rule and the nuclei, composed of chromatin lumps or a network, rarely with definitely arranged chromatin blocks along the peripheral. There was a peculiar cell, rather infrequent, and apparently one of young form; the nucleus consisted of 4-6 deeply stained triangular or quadrangular chromatin blocks, connected by fine strands, but without any membrane. The neighboring edges of these blocks did not always fit well together so that the outline of the nucleus did not form a regular surface. Often there was no nucleolus in these cells. The cytoplasm was often so small that it was difficult to see it, and in most cases it was present at one or both ends of the cell and sometimes abundantly; it was free from granulations. Another type more numerous had a thin nuclear membrane, often defective in places, the chromatin blocks being more numerous and of various forms although mostly trapezoidal with the top projecting inward. As a rule there was a nucleolus in the center, and often abundant cytoplasm, with little granulation. There were numerous intermediate forms between these types, which may be looked on as young cells that develop into the usual type by the production of a thicker nuclear membrane and the rearrangement of the chromatin blocks.

Pappenheim[22] identified the large cell described by Hodora (polyeidocyte) as identical with Schridde's[23] lymphoplastic plasma cells. This cell is of varying shape, has a small amount of cytoplasm and a large vesicular nucleus, usually central, the cytoplasm staining rather lightly. Schridde observed his cell in a study of the hyperplastic tonsil and regarded it as different from the usual plasma cells, which differs from lymphoblasts in having a cytoplasm that stains intensely and in the presence of a perinuclear halo with an eccentric position of the nucleus. He identified his cell with lymphoblasts by demonstrating with his special stain certain perinuclear granules in both these cells. Hertz,[24] discussing the literature, advanced the view that these two types are different, Hodora's cell being of the nature of a splenocyte while Schridde's cell is a lymphoblast. In the spleen of rabbits with an experimental anemia, he found in the interfollicular tissue a large cell which he called splenocytoid plasma cell and which he regards as identical with Hodora's cell. He does not regard these cells as mature, but as capable of developing into the typical plasma cell. In the pulp he found an intermediate type with a more or less oval form, eccentric nucleus, basophil cytoplasm and perinuclear halo, which he regards as a genuine plasma cell in process of formation. He did not find any of Schridde's cells in the germinal center, but he observed a cell with deeply stained cytoplasm, with on the whole a central nucleus, often without a halo, which he regards as an early stage of Schridde's lymphoblastic plasma cell. He calls it plasmacytoid lymphoblast. Evidently Herz believes that various larger types of cell may form typical plasma cells, but he does not seem to have observed the latter (Marschalko's type) with transitional forms in the germinal center; he did find, however, large numbers of the small type in the outskirts of the malpighian bodies, together with a smaller number in follicles which seem to have wandered in from the pulp.

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[22] Fol. haemat., 1911, 11, p. 170.

[23] Zeiglers Beitr., 1907, 41 p. 213.

[24] Fol. haemat., 1912, 13, p. 177.

Sleginger[25] distinguished four kinds of cells in a leukemic lymph node: (1) those with a small nucleus, with irregularly arranged chromatin, little or no cytoplasm, some having a deeply staining nucleus; (2) those with an oval or round body and eccentric nucleus with a distinct nucleolus; (3) cells with well marked granular cytoplasm, a slight perinuclear halo, the nucleus large, usually eccentric; (4) those with a large body, the vesicular nucleus often assuming a mulberry form and usually with a distinct nucleolus. He observed intermediate forms of all kinds. He does not regard the peripheral arrangement of the chromatin masses as a necessary characteristic of plasma cell and believes that Unna's plasma cell and Marschalko's cell are the same cell under slightly different forms, the small type of plasma cell developing probably from the larger.

Most authors who used hematoxylin, which has a strong affinity for chromatin, have had more or less success in tracing cell development. By means of this stain, and being able to call forth plasma cells at will, I have been able to follow their development in the spleen. Both types appear to be distinct, the large type never changing into the Marschalko type or typical plasma cell. In the germinal center I could distinguish a cell with a round or oval nucleus staining more deeply than in the case of lymphoblasts. The smaller nuclei stained more deeply with coarse chromatin masses, the larger nuclei being more vesicular, a relationship better demonstrated by double staining. Usually these cells had 1-2 and sometimes 3 nucleoli, the larger nuclei being about 8 mikrons wide and 10 or more in length; the cytoplasm was markedly granular, slightly basophil, staining red by the Unna-Pappenheim method, the nucleus nearly neutral and without any halo, as a rule. Mitotic figures were seen frequently. These cells correspond to the plasmacytoid lymphoblasts of Hertz as to character and location and to the Hodora type as to morphology but not location. I saw these cells frequently in the pulp and about the follicles as well as in the germinal centers in pathologic spleen. It may be recalled that Hodora saw them in the normal spleen in man while Hertz found them in the germinal centers in rabbits with experimental anemia and Schridde found them in the germinal centers in the hyperplastic tonsil. I fail to find any such cells in the germinal centers in the normal guinea-pig spleen as Hodora did in man. The lymphoblasts in the germinal center seems to be able from stimulation or otherwise to produce the large type of plasma cell with eccentric nucleus and perinuclear halo. In my experiments I found also large plasma cells in the pulp of the spleen with varying amounts of cytoplasm as well as other variations, all being apparently intermediary or developmental stages of the same cell. Cells corresponding exactly to Schridde's type were relatively few and usually less granular than the ones in the germinal center. Usually these cells were poor in chromatin, the nucleus being vesicular, although some had a fairly rich network of chromatin as well as isolated masses. As in the germinal centers, the smaller the size, the deeper the stain and the larger the size, the more vesicular the nucleus. Sometimes there was a pavement-like arrangement of the chromatin blocks with light lines between them, but a central nucleolus was seen only occasionally, the blocks

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[25] Virch. Arch., 1902, 169, p. 428.

being commonly arranged irregularly. Such nuclei are much smaller than the vesicular, the cytoplasm varying in amount, often being abundant. This form of cell appears after mitotic division of large lymphocytes, the young cells first showing deeply stained chromatin masses and being of irregular outline, which later becomes round or oval, when segmentation of the chromatin takes place, often with isolation of the nucleolus. Usually the segmentation occurs irregularly, without the formation of pavement-like nuclei, the chromatin forming a network or irregular masses. In further development the nucleus grows in size at the same time as the chromatin diminishes in amount so that eventually the nucleus becomes vesicular. In cells with abundant cytoplasm there may be a well developed perinuclear halo even in the earlier stages. In this type consequently the tendency to the typical nucleus ('radkern') is not marked at all. In the course of the development of the vesicular nucleus 16-20 small chromatin granules were sometimes seen inside the nuclear membrane.

There are, then, distinct and easily traced stages in the changes of the nucleus after division until it assumes vesicular form.

The Marschalko type of plasma cell appears to develop from small lymphoid cells with a deeply stained nucleus and a pink perinuclear ring and but little cytoplasm. By nuclear segmentation and growth of cytoplasm plasma cells are formed. At first crevices appear in the nucleus along which the chromatin eventually splits to form irregular blocks. At this time there is no nuclear membrane, but this appears a little later, at the same time as a nucleolus forms in the center. The chromatin gradually disappears from the central area and the crevices widen so that a typical nucleus forms to give way later to irregular masses of chromatin, 5-8 in number along the nuclear membrane. In this way more or less atypical plasma cells develop from typical forms. There is no definite rule as to the number of crevices and the chromatin masses vary in size. Atypical cells of this type are common in the spleen while the true Marschalko cell is rather rare. In the early stages there is little cytoplasm, but this develops rapidly, with usually indistinct granulation which becomes more marked later. In the young cells there may be well marked prolongations of the cytoplasm while in the older the margins seem rather thin and even vacuolated.

Authors differ as to whether plasma cells multiply by mitosis or amitosis. I have observed both forms, and the lymphocytic type of plasma cells may arise directly from lymphocytes by division. The large types of plasma cells increase mostly by mitosis; in the germinal centers amitosis of the large cell was not seen, but amitosis of small lymphocytes was common. By amitosis, plasma cells as well as lymphocytes often are divided unevenly, there being much cytoplasm at one pole and hardly any at the other. During and after mitosis both forms of cells may show a distinct granulation in the cytoplasm but this tends to disappear.

It is difficult to understand the origin of the densely stained lymphocytoid cells, whether by immigration or by local production either from lymphocytes by division or as the result of some reactive change in old cells. Judging from the appearances in the liver and the lung these cells would seem to arise from cell division in loco, but I have failed to observe any definite or marked indications of cell division immediately after the injection of the streptococci. It seems that they arise from increased activity on part of the lymphocytes as a result of stimulation by the streptococci. The so-called plasmacytoid lymphoblasts also arise, soon after injection of streptococci, from existing lymphoblasts and form plasma cells by mitosis.

These are my ideas in regard to the development of plasma cells under the conditions outlined. As stated, the lymphocytic small type and the large type, when viewed from the nuclear development, have no connection. Each produces only its kind. As Schlesinger says, we cannot at present tell whether the small lymphocytes are able to produce the large lymphocytes when influenced by special stimuli or otherwise. In the same way the small type of plasma might be conceived as able to produce large types when stimulated to repeated division, but there is no evidence that fully differentiated cells can change from one type to the other. It is possible that the large type may become small and to all intents and purposes be lymphocytes, by loss of cytoplasm and nuclear contraction, but in such a case it would be difficult to account for the changes in the structure of the nucleus, and I have not encountered any apparent transitional forms of this kind. Schridde says that he has not seen lymphocytic plasma cells develop from lymphoblasts, which Pappenheim thinks is possible. I have already stated that there seems to be no connection between the plasmacytoid lymphoblasts of Hertz and the small lymphocytic cells in and about the germinal center, as would be expected from his view. The change in the dense chromatin with course granules to the finer occurs generally in the development of cells. Taking all these points into consideration, I am unable to agree with those that advocate that Marschalko's cell type is derived from the large type of plasma cells. The cells of each type develop as they are, the chromatin undergoing reduction.

It is interesting that all the lymphocytes in the spleen, even in the follicles, should react in the same way after the injection of streptococci. In normal tissue the nuclei of lymphocytes are usually irregular and wrinkled, the chromatin being distributed as irregular granules, but after the injection the nuclear membrane is stretched out in smooth spherical or oval form and the chromatin arranged regularly in coarser blocks as it is in plasma cells, with a purple tinge. In the normal spleen and lymph nodes such cells are seen occasionally. Pappenheim[26] states that while lymphocytes and plasma cells may be isomorphous and isochromatic they nevertheless are heterogeneous elements. Leaving aside the question whether they are heterogeneous or not, it certainly is true that the lymphocytes and plasma cells, after the injection of streptococci, react isochromatically.

In the lymph nodes in various parts of the body, in the walls of the intestines, stomach and uterus, the lymphocytes reacted in the same way as in the spleen at the same time as there was an increase in the number of plasma cells.

Normally, plasma cells are often present in moderate numbers in lymph nodes as well as in the intestinal submucosa, being usually of the small lymphocytic type with a typical nucleus with a halo. After the injection of nonhemolytic streptococci, however, they increased in number and stained more deeply and distinctly, and this was especially true with regard to the uterus and intestine. In the intestinal submucosa plasma cells developed in typical form, with a large amount of cytoplasm, round or oval in shape. In places where the cells were closely packed they were irregular in shape and size. In Peyer's patches and solitary nodes the plasma cells were more of the type of the plasmacytoid lymphoblasts of Hertz or Hodora's pseudoplasma cell. Typical large cells but not with typical nucleus were frequently seen in sections of the intestine free from lymph nodes and lymphocytes were frequently found in the epithelial layer, showing fairly typical plasma cell chromatin but little cytoplasm, the shape being determined apparently by the space they occupied.

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[26] Virch. Arch., 1901, 166, p. 424; 1902, 169, p. 372.

Hodora called the plasma cells in the normal spleen polyeidocytes, that is cells with many forms, these cells being round, oval, triangular, pyramidal, spindle shape, etc., the nucleus large, and surrounded by a small amount of weakly staining cytoplasm. Even typical plasma cells with characteristic nucleus and halo, arising after the injection of streptococci, may be of many different shapes. Since Unna characterized the plasma cell as round or oval, this has been accepted as the characteristic shape of the cells, but evidently the form may be greatly modified by the conditions about the cell.

Normally the liver does not contain typical plasma cells, only some small lymphoid cells in the perithelium of the vessels and ducts, especially the latter, with deeply stained nucleus and a small amount of irregular cytoplasm. These cells correspond to Schlesinger's type 1, found in the lymph nodes in leukemia, and they also resemble my younger forms of Marschalko's type, but they do not stain as distinctly and they were few in number. Marschalko did not find plasma cells in the normal liver, while Foa observed them in small numbers in the tissue about the ducts. After the injection of streptococci these cells appeared as plasma cells, being somewaht increased in number after 3 hours and continuing for 96 hours. In the blood vessels of the liver the number of lymphocytes increased and plasma cells often were found among them. Hence the increase of plasma cells in the perithelium may be caused in part by immigration, but most of the cells undoubtedly developed from local production.

The views of the orgin of plasma cells differ greatly. They may be grouped as follows: (1) from fixed connective tissue cell (Unna,[3] L. Ehrlich [17] and others; (2) from lymphoid cells of either blood or tissue origin or both, including mononuclear leukocytes and perithelial cells, this view being supported by many investigators (Marschalko, Krompecher,[28] Maximow, Enderlen-Justi,[29] etc.) ; (3) from lymphocytes and lymphoid cells as well as fixed connective tissue cells (Pappenheim, Almgvist,[30] and others). We see that the opinions appear to favor the origin of plasma cells from lymphocytes or lymphoid cells. The question now seems to be whether the lymphoid cell is a highly differentiated fixed connective tissue cell or a real lymphocyte. Leaving aside for the moment the question whether these cells are of blood or tissue origin, the results of my work indicate that the plasma cell develops from small lymphoid cells as well as from large lymphoblasts, in the spleen and other lymphoid tissues, as the result of a special stimulation.

Pappenheim asserts that the Morschalko type is not derived from immigrated lymphocytes but rather from local tissue cells, possibly from myeloid forms, while Unna's type is of fibroblastic nature. I wish to point out with special emphasis that in my experiments the lympho-

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[27] Virch. Arch., 1904, 175, p. 198.

[28] Zeigler, Beitr., 1898, 24, p. 163.

[29] Deut. Ztschr., f. Chir., 1901-2, 62, p. 82.

[30] Arch. f. Dermat. u. Syph., 1901, 58, p. 91.

cytes in the spleen and elsewhere showed the same reaction of the chromatin on the injection of streptococci, but that the cells in the marrow failed to react, indicating a difference in the nature of the cells, and this was found to be the case even though the cells in the marrow multiply rapidly. In the course of their multiplication the cells in the marrow had no tendency to form characteristic plasma cell nucleus. Schridde made a similar observation with a special stain. These observations do not harmonize with the suggestion of Pappenheim that possibly plasma cells are derived from myeloid cells. In my experiments plasma cells appeared within 5-10 minutes in the spleen, liver and other organs, so quickly that it does not seem likely that they were immigrated cells from the blood, although the appearance of plasma cells in the blood later was a fairly regular occurrence. At the same time these cells may have developed both from preexisting lymphoid cells and from newly immigrated lymphocytes from the blood. As stated, Hertz regards his splenocytoid plasma cells as coming from splenocytes; in my experience the large type was often seen in the capillaries and sinuses of the spleen and resembled somewhat the splenocytes. However, plasma cells are not associated in phagocytosis of streptococci and red blood corpuscles, whereas splenocytes are very active in that respect. In experiments in which I injected India ink before or after streptococci, no phagocytic plasma cells were seen, even when granules were lying around plasma cells. Most authors agree that the plasma cell is not phagocytic but Marschand [31] describes the phagocytosis of lepra bacilli by these cells. Goldmann [32] distinguishes plasma cells from other cells because they are not stained vitally pyrrhol blue and Kiyono [33] separates them from other cells because they do not stain vitally with carmin. Consequently, it is difficult to accept the view of Hertz that plasma cells are derived from splanocytes. It is more likely that plasma cells develop from lymphocytes rather than from fixed connective tissue cells.

IV. LEUKOCYTOSIS FOLLOWING THE INJECTION OF KILLED NONHEMOLYTIC STREPTOCOCCI

In the last years of the previous century the changes in the number of leukocytes in the blood following the injection of protein substances, bacteria and their products, as well as organ extracts, were studied extensively. Lowit [34] expressed the idea that the leukocytes in the blood are easily destroyed as the result of the injection of foreign substances and that this destruction is followed by an overflow of leukocytes from the blood-producing organs, the chemotatic influence of the injected substances being of little or no importance. According to this view, a primary leukolysis occurs followed by a secondary leukocytosis. Lowit regarded the leukocytosis following blood letting, in inflammation, in digestion, etc. as the expression of an overcompensation following leukocytic destruction. Schulz,[35] however, observed that after the injection of various substances the leukocytes would be distributed irregularly in the body, and on this account he could not accept the view of hypoleukocytosis and hyperleukocytosis. Goldscheider and Jacobs [36] observed that in the stage of leukopenia the leukocytes gather in the internal organs, especially the lungs, and they attributed this to chemotactic action of the substances infected and explained the subsequent hyperleukocytosis on the same basis. In 1904 Arneth [37] noted that the neutrophil leukocytes undergo morphologic changes in infectious diseases, and he classified these leukocytes into 5 types according to the number of the nuclear lobules. He advanced the view that the neutrophil leukocytes were destroyed as a result of the infection. In my experiments with India ink and with physiologic salt solution I noted usually a slight degree of stimulation in the lung during the period of leukopenia. This usually lasted from 10 to 30 minutes after the injection, sometimes as long as 60 minutes and was then followed by a leukocytosis that reached its maximum after 3 to 4 hours. When large quantities of ink were injected there resulted a more marked destruction of cells without leukocytosis, the pseudo-eosinophil cells being replaced by a new immature cell some time after the injection, showing distinctly that destruction, as well as chemotaetic influence, play a significant part. After the injection of killed nonhemolytic streptococci the distribution of leukocytes in the early stages corresponded to that described by Goldscheider and Jacob, large numbers gathering in the lung; this fact accounts in some degree for the leukopenia, and the changes in the bone marrow indicate a rapid mobilization and proliferation of cells. Muir [38] observed a marked proliferative progress in the marrow in hyperleukocycosis with decrease of polymorphonuclear cells while Rubinstein[39] found an increase of lymphoid cells, myelocytes and transitional forms, the polymorphonuclears showing a decrease. These authors interpret the decrease of polymorphonuclears in the marrow in this instance as the result of rapid transfer of cells to the blood. Up to this time there was usually not observed any accumulation of polymorphonuclears in the marrow in infection, spontaneous or exeprimental, but this probably is due to its manifest nature and its occurrence shortly after the introduction of the foreign substances.

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[31] Verhandl. d. Deutsch. Path. Gesell., 1913, 16, p. 5.

[32] Beitr. f. klin. Chir., 1909, 64, p. 192.

[33] Folia haemat., 1913, 15, p. 282; Die vitale Karminspeicherung, Jena, 1914; Nisshin-Igaku, 1914, 4, p. 917 and 1113.

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[34] Studies zur physiologie und pathologie des Blutes und der Lymphe, Jena, 1892.

[35] Deutsch. Arch. f. klin. Med., 1893, 51, p. 234.

[36] Ztschr. f. klin. Med., 1894, 25, p. 373.

[37] Die Neutrophilen Weissen Blutkörperchen, Jena, 1904.

[38] Jour. of Path. and Bacteriol., 1900-01, 8, p. 161.

I shall discuss the changes in the leukocytes in the blood after the injection of killed nonhemolytic streptococci. Some of the questions to be solved if possible are the fate of the leukocytes that are destroyed and whether new and special forms of cells would appear as after the injection of India ink. The question as to how long the cocci remain in the blood stream, inside as well as outside the leukocytes, also presents itself for consideration.

In the experiment another strain of nonhemolytic streptococcus was injected. Otherwise the same procedure was followed and blood was taken from the right ventricle of the heart at various intervals after the injection with a fine needle and syringe. Fresh counts were made and also stained preparations. The total number of leukocytes was obtained by getting the average of ten large square fields from both sides of Turks' double chamber chemocytometer. Wright stain was used for differentiation and hematoxylin and eosin stains were used to determine Arneth's counts. The same suspension of streptococci was used throughout the experiment, the suspension being kept in the icebox and warmed to about 38 C. each time before injection.

The results obtained in these experiments were not consistent. Apparently unknown factors requiring further study played a part. The observations recorded in Table 3 were, however, so consistent as to require no further observations.

As to the total number, it gradually increased reaching a maximum about 3 hours after injection and then fell gradually in the case of guinea-pig 1 in which there was no fall immediately after the injection as expected. In guinea-pig 2 the number increased rapidly and returned to normal after 6 hours. In guinea-pigs 3 and 4 death occurred at the end of 1 hour when there was a distinct leukopenia. These animals remained on the holder for 30 minutes after the injection. Lowit noted a decrease in the number of leukocytes as soon as the animal was placed on the holder but did not examine the blood of the heart. He also found that lowering of the temperature of the body reduced the number of leukocytes and Goldscheider and Jacob believed that such lowering accounted for the fall in the leukocytes rather than placing the animal on a holder. The other animals in my experiments showed no decrease and very little increase in the period concerned. When we consider the preservation of the suspension of the streptococci, the possibility arises that products may have passed into solution that were toxic for the animals, and on this account the cocci were washed before injecting guinea-pigs 5 and 6, the results not differing very much from those obtained in animals 1 and 2. In none of these experiments except in that performed on animal 4 was there any marked fall in leukocytes in the heart blood within 10-30 minutes after the injection, but there was a more or less irregularly occurring increase in the number.

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[39] Ztschr. f. klin. Med., 1900-01, 42, p. 161.

The differential count established a decrease in the large mononuclear leukocytes throughout the period under observation and of the polymorphonuclears for a short time after the injection when the number rose again reaching normal in about 24 hours. The lymphocytes, however, increased in number soon after the injection in most cases, and the polymorphonuclears increased after 3 hours in every case, returning to normal at the end of 24 hours. The fact that there was no diminution of leukocytes immediately after injection may have been due principally to increase in the number of lymphocytes. The polymorphonuclears and large mononuclears were usually fewer than normally 10 minutes after the injection. In 3 hours, however, the polymorphonuclears had increased beyond their normal number while the large mononuclears tended rather to decrease in number. In my work with India ink I observed that the large mononuclears do not usually enter the blood stream as such but develop mainly from smaller cells as asserted by Gulland and others, and that one or two days are required for their full development when their phagocytic activity is at its height. They may accumulate in the lungs shortly after the injection as the polymorphonuclears do. If not destroyed in the lungs and if they reenter the circulation, the number of mononuclears should be restored promptly, but their number continued low for 24 hours. Where do the polymorphonuclears come from when their number is increased beyond the normal? Are they newly formed cells produced in the marrow, or do they come from the lung? The leukocytes containing bacteria leave the lungs early and this consequently may be the cause of the rise of such cells in the blood. The histologic examination showed that at this time polymorphonuclears accumulate in the internal organs, such as the liver and spleen, and the increase of these cells in the blood in the right ventricle suggests that they come not only from the lung but also from the blood-forming organs. On the whole, it seems to me that leukopenia and hyperleukocytosis may be caused by the same factor, such as a local positive chemotaxis leading to accumulation in the lung and later in the liver and spleen, as shown in this work, creating a demand for leukocytes from the blood-forming organs and a diminution of leukocytes in the blood. The chemotactic influence affects not only the reserve leukocytes in the marrow, but also stimulates the myelocytes to produce new cells and thus hyperleukocytosis develops. As the streptococci that have been taken up by leukocytes undergo disintegration, substances are set free that may have chemotactic effects and stimulate cell production. If the leukocytes are withdrawn from the blood by the presence of widely spread substances, all organs should receive leukocytes, but my results show that leukopenia as well as hyperleukocytosis are associated with an irregular distribution of the leukocytes owing to their being attracted to places where the streptococci localize. Thus, as already stated, there is a large number of polymorphonuclear leukocytes in the spleen normally, but immediately after the injection of streptococci there was a distinct diminution of leukocytes in the spleen. This occurred also in the bone marrow. The leukocytes undoubtedly pass to the lungs where streptococci accumulated quickly after the injection, as shown especially by the increased number of leukocytes in the earliest stages after the injection. Buchner[40] and others have shown that bacterial protein and cellular protein have a positive chemotactic effect, and it would seem reasonable to believe that the killed streptococci when injected into the blood may liberate similar substances. If leukocytes and other cells undergo destruction, chemotactic substances may be set free and give rise to leukocytosis. In the case of the animals injected with streptococci, cocci and leukocytes accumulate in the lung very quickly and in this respect the result differs greatly from the result of the injection of a simple foreign body, such as a cinnabar without gelatin. It may be pointed out that

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[40] Berl. klin. Wchnschr., 1890, 27, p. 673; ibid., p. 1084.

TABLE 3

ANALYSIS OF LEUKOCYTES

Guinea-Pigs	Leukocytes	Before Injection	Time After Injection								Remarks
			10 Minutes	30 Minutes	1 Hour	3 Hours	6 Hours	12 Hours	24 Hours	48 Hours	
1. 350 gm. injected with 2.3 c c. suspension 2 days old	Total number	11,976	13,280	14,780	17,460	23,840	17,422	18,700	10,320	8,460	
2. 300 gm. injected with 2 c.c. suspension 3 days old	Total number	5,780	10,110	11,500	10,660	7,140	5,480	5,500	6,000	......	A moderate number of free cocci at 10 minutes, none at 30
	Mononuclears, %	20.6	10.9	8.2	15.2	10	8.7	16.6	6.2		
	Lymphocytes, %	61	75	75.3	58.7	30.4	31.9	29.1	69.1		
	Polymorphonuclears, %	15.2	13.2	15.5	23.4	58.4	59	53.6	24.3		
	Other cells, %	3.1	0.8	0.9	2.6	1.1	0.4	0.6	0.3		
	Arneth's count I	19	......	3	13	44	40	19	10	......	At 3 hours 98% pseudos replaced by cells which died in about 20 hours
	II	10	......	12	18	44	38	34	33		
	III	10	......	28	37	11	20	36	37		
	IV	28	......	34	25	1	2	10	16		
	V	35	......	23	7	0	0	1	4		
	Phagocytic mononuclears, %	.....	8.7	5.4	2.8	0	0	0	0		
	Phagocytic polymorphonuclears, %	.....	44.2	22.2	11	2.6	0	0	0		
3. 280 gm. injected with 2 c c suspension 4 days old	Total number	6,466	7,590	5,820	2,120	......	......	......	......	......	Moderate number of cocci among blood platelets at 10 minutes, hardly any at 30
	Mononuclears, %	17	17.6	11.1	12.7						
	Lymphocytes, %	37.8	53.4	44.4	38.7						
	Polymorphonuclears, %	42	26.1	40	43.9						
	Other cells, %	3.1	2.8	4.4	4.6						
	Phagocytic mononuclears, %	.....	9.3	4.4	9						
	Phagocytic polymorphonuclears, %	.....	23.3	7.7	28						
4. 290 gm. injected with 1.5 c c suspension 5 days old	Total number	4,884	2,820	2,140	1,253	......	......	......	......	......	A fair number of cocci among blood platelets at 10 minutes, only a small number at 30 minutes
	Mononuclears, %	30.2	18.7	9							
	Lymphocytes, %	37.8	49.9	61.8							
	Polymorphonuclears, %	29.3	27.3	22.7							
	Other cells, %	2.6	4	6.4							
	Phagocytic mononuclears, %	.....	2.4	0							
	Phagocytic polymorphonuclears, %	.....	23.3	40							

5. 260 gm. injected with 1.5 c c suspension 6 days old after washing the cocci	Total number	4,300	6,500	5,520	5,520	5,350	2,420	3,060	3,680	......	Moderate number of free cocci among platelets at 10 m., none at 30. No peculiar cells after 1 hour; at 3 hours all pseudoeosinophils were replaced by peculiar cells; at 12 hours cells mostly of normal type with some transitional forms; at 24 hours peculiar cells rarely seen
	Mononuclears, %	34	13.9	12.4	12.1	17.7	15.4	10.3	14.4		
	Lymphocytes, %	21.6	54.7	46.7	58.3	23.6	30.9	41.3	45.3		
	Polymorphonuclears, %	36	27.8	34.7	21.2	52.9	47.7	47.1	34.1		
	Other cells, %	8.3	3.5	6.1	8.3	5.7	5.9	1.2	6.1		
	Phagocytic mononuclears, %	.....	0	0	0	0	0	0	0		
	Phagocytic polymorphonuclears, %	.....	23.2	29.6	21.4	2.7	0	0	0		
	Phagocytic eosinophils	.....	2.1	0	0	0	0	0	0		
	Arneth's count I	3	......	......	......	43	41	16	19		
	II	11	......	......	......	44	36	32	31		
	III	21	......	......	......	11	20	38	40		
	IV	27	......	......	......	2	3	12	9		
	V	38	......	......	......	0	0	2	1		
6. Suspension 7 days old	Total number	5,420	7,400	6,380	6,850	7,800	3,984	......	4,200	......	Only a small number of free cocci at 30 min. No special form of polymorphonuclear at 1 hr., but at 3 hrs. 95% were special type and at 6 hrs. transitionals and small type still abundant, but at 24 hours nearly all cells were of the usual type
	Mononuclears, %	15.3	......	4.2	4.4	2.1	1.5	......	6.3		
	Lymphocytes, %	55.8	......	74.4	69.6	55.7	30.8	......	65.3		
	Polymorphonuclears, %	15.3	......	20.7	24.3	40.5	66.6	......	27.4		
	Other cells, %	0	......	0.6	1.6	1.6	0	......	0.9		
	Phagocytic mononuclears, %	.....	......	0	0	0	0	......	0		
	Phagocytic polymorphonuclears, %	.....	......	34.5	24.4	3.5	1.2	......	0		
	Phagocytic eosinophils	.....	......	3.1	0	0	0	......	0		
	Arneth's count I	2	......	4	11	37	26	......	7		
	II	9	......	15	17	41	40	......	21		
	III	32	......	36	45	20	25	......	31		
	IV	17	......	30	22	2	7	......	30		
	V	18	......	15	5	0	2	......	11		

the loss of streptococci in suspension of the ability to stain by Gram's method may be associated with the liberation of chemotactic elements. Certainly during the course of phagocytosis, chemotactic substances may be freed by cell destruction and otherwise. Taking these factors into consideration, it seems safe to conclude that the changes in the distribution and number of leukocytes are the result of chemotactic effects.

Among the interesting changes that follow the injection of killed nonhemolytic streptococci is the production of a new kind of cell. Not only were the pseudoeosinophils displaced to the left in Arneth's table, but they multiplied rapidly. With the immature forms, including Papenheim's metamyelocytes, similar to those that I noted after the injection of India ink, this change occurred as quickly as three hours after the injection. In animals 2 and 5 this blood picture continued until about the sixth hour; by the twelfth hour the pseudoeosinophils had decreased and a smaller type with transitional forms then predominated, and after 24 hours normal conditions were practically reestablished. In animal 6, however, the return to the normal seemed to have taken place more rapidly. This cell also took up cocci. The changes in the blood correspond well to the changes in the marrow. As stated, the myelocytes during the early stages after the injection were enlarged and there appeared well lobulated polymorphonuclears in the marrow after 24 hours and later. Pappenheim[41] classifies the myelocytes in young mother cells and young micromyelocytes, the latter developing from the former and becoming transformed into the usual polymorphonuclear cells; the mother myelocytes appear in the blood not only in chronic myelogenous leukemia, but in acute cases polymorphonuclear giant leukocytes are found in the blood. The large myelocytes in the marrow in guinea-pigs injected with streptococci corresponded to the mother myelocytes and polymorphonuclear giant leukocytes were also seen. Pappenheim and Szecsi,[42] as well as Isaac and Moeckel,[43] observed similar cells after saponin injection. We see then that within 3 hours after the injection the reserve cells in the marrow became exhausted and that there appeared a large myelocyte and immature cells in the blood in large numbers. A complete change in the morphology of the leukocytes took place in 3 hours, showing what a large number of cells were demanded, how rapidly they perished and how freely their loss was compensated, the ability to supply normal cells being exhausted and immature cells supplied instead. It is probable that the phenomena observed in connection with the leukocytes also took place with respect to the endothelial cells of the liver and spleen, as indicated by the irregular changes observed in their shape, staining and distribution.

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[41] Atlas d. Menschlichen Blutzellen, 1911-12, Suppl. p. 96.

[42] Fol. Haemat., 1912, 13, p. 25.

[43] Ztschr. f. klin. Med., 1911, 72, p. 232.

In normal guinea-pigs the nuclei of the leukocytes are much more lobulated than in man and they belong principally to classes 3, 4 and 5 in Arneth's scheme. Thirty minutes after the injection and more noticeably one hour later there was a displacement to the left; at 3 and 6 hours after the injection this position was entirely reversed, class 5 being lacking in most cases. As the table shows, classes 1 and 2 constituted 78 to 87% at 3 hours and 66 to 77% at 6 hours. In all cases the maximum was reached at 3 hours and in no case was the normal position regained after 24 hours; animal 6 showed the most rapid return to normal. Arneth was hardly able to recognize any changes from the normal in digestive leukocytosis; after baths and even in infectious diseasees there was only a slight displacement if any in light cases My tables shows that even when many bacteria are introduced at once so that many cells are required for defense, the bone marrow is still able to furnish leukocytes of the usual type, provided it is in normal condition. The marrow always has a considerable reserve of polymorphonuclear leukocytes. When the demand is urgent and continuous the reserve is soon exhausted and younger forms are sent out with a corresponding displacement to the left in Arneth's scale, and eventually special types of cells, such as the large polymorphonuclears with basophil granules, appear. Apparently it takes a little time after cell division before a polymorphonuclear leukocyte reaches its normal and usual form. It is possible that immature cells, which usually are found in the marrow under such conditions, may ripen in the blood, although it appears that usually such types are soon destroyed. Frequently I encountered large cells with fantastically shaped nuclei, sometimes partly segmented, resembling megakaryocytes, and these no doubt are extreme examples of abnormal newly formed leukocytes. The changes found in the leukocytes after the injection of streptococci may be due to fluctuations and changes in chemotactic substances in the blood. We do not know the fate of such substances, whether they are discharged by certain organs or assimilated; we know only that the cocci disappear completely within 72-96 hours after the injection with gradual changes in form and staining reaction, mostly within cells and that at the same time many cells undergo disintegration. In the meantime, the leukocytes in the blood rise and fall as the balance between destruction and production varies and in any case the appearance of immature leukocytes indicates an acute demand either on account of a general destruction of leukocytes or on accout of their being attracted in large numbers to some particular place. The rapid changes in the leukocytes soon after the injection shows how powerful the chemotactic influences at that time were.

In view of what has been stated, it appears that in infectious diseases a hyperleukocytosis or a chronic leukopenia, with normal polymorphonuclears, indicates that there is a reserve supply of leukocytes in the marrow; in other words, that the prognosis seems good; but an acute hyperleukocytosis with many immature leukocytes with marked displacement to the left indicates that the demands on the marrow are approaching the limit of its powers. This would be still more strongly indicated in case an acute leukopenia, affecting especially the polymorphonuclears, should develop. At this point it may be suggested that in acute infectious diseases careful examination of the blood by Arneth's method is indicated, especially when vaccine treatment is used, because the injection of killed bacteria or their products at short intervals may serve to increase the exhaustion of leukocytes.

In these experiments it was assumed that the streptococci that were injected would remain free in the blood for about 30 minutes, but it was found that they quickly became enclosed within masses of platelets and apparently rapidly reduced in number. As the table shows, streptococci were found within leukocytes in most cases for as long as 3 hours; in animal 6 there were cocci within the leukocytes 6 hours after injection. It is possible that cocci remain free in the blood in small numbers for a longer time because so many phagocytic cells were attracted to certain organs; even derivatives of mother myelocytes contained cocci. In some of the animals mononuclear cells were found to contain cocci. Eosinophil leukocytes, which do not take up India ink granules, frequently contained cocci.

SUMMARY

On injection of homogenous suspensions of nonhemolytic streptococci into the jugular vein of guinea-pigs, the cocci first accumulate principally in the lung, a comparatively small number only passing through this organ into the general blood stream, whence they are taken up by endothelial cells in the liver and by endothelial and other cells in the slpeen. Injection into the left ventricle and into the portal vein also resulted in cocci collecting to some extent in the lung. This accumulation of cocci in the lung seems to be largely mechanical and the result of the formation of masses of cocci in the blood on the one hand and the narrowness of the capillaries in the lungs on the other hand.

In the lung the cocci are taken up principally by polymorphonuclear leukocytes and to a much less degree by mononuclear and endothelial cells. The rapid collection of leukocytes in the narrow pulmonary capillaries hinders the dispersion of bacteria into the blood current. The leukocytes are actively phagocytic and within about 10 minutes after the injection apparently all the cocci are taken up; in from 30 minutes to 1 hour the phagocytic leukocytes leave the lungs, the majority going to the liver and spleen, where there is a great increase in such cells up to about 6 hours after the injection. Many cocci are digested in the cells in a short time, but it may require from 72 to 96 hours before all the cocci disappear. During this time the cocci in the cells become smaller, staining irregularly, and finally forming red granules.

The phagocytic action of endothelial and mononuclear cells in the lung is not marked and does not constitute a factor of any great importance in the elimination of the cocci, but in the liver the endothelial cells are very active in phagocytosis, more so by far than the cells of the spleen. On account of its volume and the phagocytic activity of its endothelial cells the liver must be regarded as the most important place of elimination of the bacteria, the spleen probably being next in importance. Of the leukocytes in the blood the polymorphonuclears are by far the most active and undoubtedly play an important part in the digestion of the cocci. Comparatively speaking, the mononuclear and eosinophil leukocytes are less active.

The fixed cells of the marrow are not as active in phagocytosis as those of the spleen, but in the marrow an active supply of new leukocytes develops very soon after the injection, that is within 5 to 10 minutes, and in 1 to 3 hours there is abundant evidence of new formation of leukocytes from myelocytes.

The endothelial cells of the blood vessels elsewhere than in the organs and tissues mentioned do not appear to act as phagocytes except in the case of the suprarenals and the cortex of the kidney, especially the glomeruli, but the activity here does not seem to be marked. The salivary glands, pancreas and lymph nodes do not seem to participate actively in the elimination of the cocci, which are found only within the leukocytes in these tissues. There is no special relation between the cocci and the walls of the stomach. The endothelial cells of the capillaries of the liver, the lung and also to some extent of the suprarenals and kidneys seem to take on increased activity soon after the injection. This was marked particularly in the liver in which the endothelial cells changed their shape and increased more or less in size, the nuclei staining deeply, the changed cells having a rather irregular distribution. In the spleen there was general proliferation of the reticular cells and of the cells in the malpighian bodies; here a degenerative form of lymphocytes with pykosis or karyorhexis appeared. In the marrow the megakaryocytes also fell into pyknosis on the one hand, others showing mitosis. Soon after the injection plasma cells of various types appeared in the spleen, lymph nodes, and the submucosa of the gastrointestinal tract, uterus, and in other places (see part 2).

The outstanding features of the cellular reaction after the intravenous injection of nonhemolytic streptococci are the accumulation of phagocytic polymorphonuclears in the capillaries of the lung, the liver and spleen; the great leukocytogenic activity of the marrow; and the phagocytic activity of the endothelial cells of the liver and spleen, and of the polymorphonuclears in the blood.

In the spleen were found degeneration forms of the nucleus (Flemming's tingible body), derived in this case from small and large lymphocytes by pyknosis and karorrhexis. Pyknosis apparently resulted from a perinuclear or net hyperchromatosis while karyorrhexis affected chromosomes after cell division, the first step being the formation of the so-called mulberry nucleus. In some cases, however, cells disintegrated that were not in process of division, the nuclei undergoing swelling and assuming budded forms at the same time as the cells contained cocci. These appearances coincide with the appearances that develop after injection of India ink. In the pyknotic group the cells were usually situated within large phagocytes. Irregular disintegration forms appeared also in lymph nodes, intestinal walls, the liver and elsewhere.

In the spleen of the normal guinea-pig there often is a small number of plasma cells; these cells sometimes have a typical plasma cell nucleus, but more usually the cell body is rather flat and does not stain well. After the injection of nonhemolytic streptococci, however, plasma cells became abundant and appeared typical in shape and staining reaction, most of them being of the lymphocytic type (Marschalko), but a larger type also appeared in the pulp and in the germinal centers. The plasma cell of the former type was present in small numbers in the follicles and germinal centers. The large type corresponds to the plasmacytoid lymphoblast of Hertz and to Hodora's pseudoplasma cell, but not to Schridde's lymphoblastic plasma cells. In the pulp were various forms of this large type but there were generally few well developed Schridde's cells.

Cells of the Marschalko type developed from lymphoid cells, the nuclei of which stain homogeneously and separate into segments, without nuclear membrane. In the course of this segmentation the nucleolus often comes to occupy the center. Later a nuclear membrane appears and the segments arrange themselves along its inner surface, a more or less typical nucleus being produced. This change appears to represent a reaction on the part of the lymphocytes.

Plasma cells of the small type multiply by mitotic as well as by amitotic division, while a large type multiplies principally by mitosis.

The chromatin in the large type is scanty, but a short time after division it assumes its usual arrangment; in both types of plasma cells the younger have more chromatin in the form of blocks and masses that stain deeply. As the cell grows the nucleus becomes more vesicular in appearance. There is no indication that these two types of plasma cells change from one into the other.

The plasma cells in the spleen have a relatively small amount of cytoplasm and may assume various shapes while those in the intestine and uterus have rather more cytoplasm and are round or oval, due to the mechanical conditions present.

As indicated, the plasma cells that appear in the spleen and other organs after injection of nonhemolytic streptococci are not the result of immigration but of local production. In the perithelium of the vessels of the liver and especially about the bile ducts occur normally lymphoid cells and 3 hours after the injection of streptococci these cells are increased, this process continuing for about 96 hours.

Undoubtedly the plasma cells sometimes observed in the blood either of the heart or of other organs were in the course of migration. It would seem reasonable to conclude that in local inflammation and tumors plasma cells may develop from existing perithelial lymphoid cells, as well as from immigrated lymphocytes.

The plasma cells did not take up any streptococci, ink granules or blood pigment, while splenocytes had strong avidity for such particles. This is in agreement with the observations of others and particularly with results of Goldmann's experiments with pyrrol blue and of Kiyono's vital stain with lithium carmin. The opinion of Hertz that plasma cells (Hodora type) are derived from splenocytes is not supported by my observations, and the indications are further that plasma cells do not come from the fixed cells of connective tissue.

Generally speaking, the lymphocytes in the spleen, lymph nodes and other organs present the same reactions on the part of the chromatin as the plasma cells, changing from being shrunken to being stretched, from staining indistinctly to staining distinctly, and with my double stain the coarser chromatin particles assumed a purple tint, particularly those along the nuclear membrane, especially in plasma cells.

Injections of a suspension of killed nonhemolytic streptococci, kept for 5 days, and of suspension of washed cocci, caused a varying degree of hyperleukocytosis in the blood of the right ventricle as early as 10 minutes after injection. At the same time, there accumulated in the lungs large numbers of leukocytes so that one would expect a general leukopenia. The hyperleukocytosis continued for about 3 hours, and then disappeared more or less gradually. The latter period of this hyperleukocytosis corresponds to the period when the number of leukocytes in the lungs decreases while in the liver and spleen the number increases. These facts indicate that not only are leukocytes attracted from the blood to the places where the cocci accumulate in largest number, but also and very promptly from the blood forming organs. The negative chemotaxis that Goldscheider and Jacob advanced as an explanation of the accumlation of leukocytes in the lungs and of the leukopenia cannot be accepted; a more reasonable explanation is that a local positive chemotaxis is produced immediately after the injection by the bacteria, as well as by the products of cells that undergo disintegration.

The large mononuclear leukocytes decreased in number immediately after the injection and were not restored within 24 hours, an observation that agrees with the view that these leukocytes do not come into the blood as they are, but rather develop in the blood, and that after having reached full growth they are destroyed easily and quickly. The lymphocytes increased promptly and then gradually decreased to normal again in about 24 hours or so. The polymorphonuclears decreased promptly but in 3-12 hours there was a marked increase and at the end of 24 hours the normal number was reached.

Disarrangement of the normal Arneth's scale began promptly after the injection and reached its maximum in about 3 hours when classes one and two constituted 78-88%. At 6 hours classes 1 and 2 constituted 66-78%. There then ensued a gradual return, but normal conditions were not regained fully at the end of 24 hours. The normal type of cell was replaced within 3 hours by an abnormally large polymorphonuclear leukocyte (mother myelocyte and metamyelocyte, Pappenheim), and in 3 experiments these cells at the end of 3 hours constituted 98, 100, and 95%. This type vanished almost wholly in 24 hours.

This condition in the blood harmonizes well with the result of microscopic examination of the marrow and appears to be the result of an exhaustion of the reserve cells in the marrow and repeated rapid cell division.

The accumulation of polymorphonuclear leukocytes in the lung may be a factor in the general leukopenia that developed after the injection of various bacteria and other substances, but it is not the only factor. Leukocytes may be destroyed so rapidly that in spite of rapid mobilization from the blood-forming organs the normal type of polymorphonuclears may be exhausted within 3 hours or so and replaced by a special type of young cell.

The lymphocytes in the blood from the right ventricle were found to increase from the first after the injection, and in this respect my results agree with those of Lowit.

Only a few phagocytic leukocytes were found in the blood 3 hours after the injection, and in 6 hours such leukocytes seemed to have disappeared entirely and no free cocci could be seen. Eosinophil cells also took up cocci.

In infectious diseases hyperleukocytosis or leukopenia is not a dangerous sign, provided the blood presents the normal leukocytic picture, but a marked displacement to the left in Aarneth's count, especially when associated with diminution in the number of leukocytes, is a sign of danger, as it means exhaustion of the leukocytogenic function of the marrow.

In severe acute infections the blood should be examined carefully according to Arneth's method, before as well as after the injection of vaccine in case vaccine treatment is used. Repeated injections of vaccines at short intervals may produce a more or less severe exhaustion of the marrow.

Explanation of Plate I

Fig. 1.—Various stages of plasmacytoid lymphoblasts (Hertz) in germinal center with a few small cells of the Marschalko type; spleen, 30 minutes after injection. Unna-Pappenheim stain. Figures 1 and 2, Leitz ocular 4, Spencer objective, oil immersion 1.8; other figures same objective but ocular Leitz 6. All figures outlined by aid of Zeiss apparatus.

Fig. 2.—Development stages of nucleus of large plasma cells after mitosis. 'A1 and A2, at the right, young Schridde type; A2, left, still younger; B, usual Schridde type; spleen, 5 minutes after injection; hematoxylineosin and Gram-Weigert stain.

Fig. 3.—A, Schridde type in pulp; B, abnormally developed vesicular Hertz type with reguarly arranged chromatin granules inside of nuclear membrane; hematoxylin-Gram double stain.

Fig. 4.—Developmental stages, Marschalko cell. A, lymphoid cell; B, faint crevices; C, developed crevices, no nuclear membrane; D, appearance of nuclear membrane; E, usual Marschalko type in spleen.

Fig. 5.—A, various Karyorrhectic degeneration forms of small lymphocytes 6 to 12 hours after injection; B, special peculiar cells in liver of rabbit with chronic septicemia; hematoxylin-eosin stain.

Fig. 6.—Various forms of karyorrhexis in large lymphocytes.

Fig. 7.—Hyperchromatosis and pyknosis of large and small lymphocytes.

Fig. 8.—Disintegration of compact homogenous nucleus of lymphocytes.

Fig. 9.—Pyknotic nuclei taken up by large phagocytes.

PLATE 1